

# High Rates of Energy Expenditure and Water Flux in Free-Ranging Point Reyes Mountain Beavers *Aplodontia rufa phaea*

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## ABSTRACT

We measured water flux and energy expenditure in free-ranging Point Reyes mountain beavers *Aplodontia rufa phaea* by using the doubly labeled water method. Previous laboratory investigations have suggested weak urinary concentrating ability, high rates of water flux, and low basal metabolic rates in this species. However, free-ranging measurements from hygric mammals are rare, and it is not known how these features interact in the environment. Rates of water flux ( $210 \pm 32$  mL d<sup>-1</sup>) and field metabolic rates ( $1,488 \pm 486$  kJ d<sup>-1</sup>) were 159% and 265%, respectively, of values predicted by allometric equations for similar-sized herbivores. Mountain beavers can likely meet their water needs through metabolic water production and preformed water in food and thus remain in water balance without access to free water. Arginine-vasopressin levels were strongly correlated with rates of water flux and plasma urea : creatinine ratios, suggesting an important role for this hormone in regulating urinary water loss in mountain beavers. High field metabolic rates may result from cool burrow temperatures that are well below lower critical temperatures measured in previous laboratory studies and suggest that ther-

moregulation costs may strongly influence field energetics and water flux in semifossorial mammals.

## Introduction

Physiological capacity to regulate osmotic exchanges with the environment strongly influences water and dietary requirements as well as habitat preferences in animals. Differences in water flux rates between species are partly influenced by the availability of preformed water in the diet and in water loss required for urinary excretion (Nagy and Peterson 1988). While numerous studies have investigated the behavioral and physiological strategies for maintaining water economy and energy balance in arid environments (e.g., Bartholomew 1964; Louw and Seely 1982), few have examined water flux and energy expenditure of eutherian mammals living in hygric habitats. One species for which wet habitat characteristics appear to be especially critical is the mountain beaver *Aplodontia rufa*. While water economy has been the subject of considerable laboratory investigation, no studies have examined it in this species under natural conditions.

*Aplodontia* are considered to be the oldest group of living rodents and the sole extant member of the superfamily Aplo-dontoidae. They are largely morphologically unchanged in the fossil record since the Miocene (Simpson 1945). Molecular studies have consistently suggested a sister relationship between the mountain beaver and the sciuridae (Adkins et al. 2001). The seven subspecies of mountain beaver (Hall 1981) are found in cool, moist climates along the West Coast of North America, from British Columbia to central California and east to the Cascade and Sierra Nevada mountain ranges. Two of the subspecies, the Point Arena mountain beaver (*Aplodontia rufa nigra*) and the Point Reyes mountain beaver (*Aplodontia rufa phaea*) inhabit small isolated areas and are considered endangered and threatened, respectively (Steele 1986).

Mountain beavers feed on a wide variety of vegetation and live in underground burrows that are usually found in moist areas with well-drained soil. The most important habitat factors appear to be cool temperatures, adequate soil drainage, abundant food supply (Beier 1989), vegetative coverage, and soft soil (Hacker and Coblenz 1993). Limits to distribution are associated with rainfall and soil characteristics that promote lush vegetation and near 100% humidity within burrows. Mountain beavers maintain fecal and food caches and practice

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coprophagia (Carraway and Verts 1993). Dramatic reductions in Point Reyes mountain beaver population numbers have been associated with fire (Fellers et al. 2004).

Investigations have revealed associations among renal structure, renal concentrating ability, and habitat (Beuchat 1996; Alkahtani et al. 2004). Previous studies have suggested that *Aplodontia* kidneys may lack the anatomical structures necessary to effectively concentrate urine to hypertonicity (Pfeiffer et al. 1960; House et al. 1963; Schmidt-Nielsen and Pfeiffer 1970). Captive mountain beavers consumed  $327 \text{ mL kg}^{-1} \text{ d}^{-1}$ , losing most of this water as urine (Nungesser and Pfeiffer 1965). McNab (1979) reported high body temperature ( $38^\circ\text{C}$ ), low basal metabolic rates, and standard thermal conductance for captive animals. In this study, we examined the water flux, energy expenditure, and hormonal regulation of renal function in free-ranging mountain beavers during a period of no rainfall. We hypothesized that free-ranging Point Reyes mountain beavers will have high rates of water flux, despite relatively low field metabolic rate (FMR), resulting in low water economy.

## Material and Methods

### *Subjects and Study Site*

All animal-use protocols were reviewed and approved by the Sonoma State University Institutional Animal Care and Use Committee. This study was conducted at Point Reyes National Seashore, Marin County, California, during April and May 2004. Mountain beavers were caught using rigid Tomahawk live traps (6 in  $\times$  6 in  $\times$  24 in) set outside burrows thought to be active. Each trap was baited with fresh apple slices and covered with burlap. Animals were anesthetized using vaporized isoflurane gas in a custom-built Plexiglas anesthesia chamber connected to a portable gas anesthesia machine. Once anesthetized, the animal was removed from the trap and fitted with a mask through which the isoflurane gas was administered throughout the sampling procedure.

### *Water Flux and Energy Expenditure*

Measurements of FMR and water flux were obtained using the doubly labeled water (DLW) technique (Lifson and McClintock 1966; Nagy 1980; Speakman 1997). The DLW technique has been validated at high rates of water flux (Van Trigt et al. 2002). After an initial 3-mL blood sample was taken from the jugular vein, 2 mL of DLW ( $^{18}\text{O}$  95APE,  $^3\text{H}$  12.3 MBq) was injected intraperitoneally. During an equilibration period of 90 min, the mask was removed, and the animal was placed back into the chamber under a decreased level of anesthesia. After 90 min, the anesthesia was increased, and a final 2-mL blood sample was taken. Animals were weighed using a digital scale ( $\pm 1.0$  g) and were marked on the chest by clipping a patch of fur and writing a number on the skin with a permanent marker. Animals were then placed at the entrance to their burrows and

monitored as they recovered from the anesthesia. Animals were recaptured 5–7 d following their initial capture and were anesthetized. The animal was reweighed, and a 3-mL blood sample was taken. The animal was monitored as it recovered from anesthesia and then was released.

The activity of  $^3\text{H}$  in each serum sample was measured in triplicate 100- $\mu\text{L}$  aliquots of serum by using the freeze-capture method (Ortiz et al. 1978). Samples were counted in 10 mL of Ecolite scintillation cocktail by using a Beckmann LS3801 scintillation counter. The activity of an injectate standard was measured in triplicate at the same time as the samples. The  $^{18}\text{O}$  enrichments of samples were analyzed in duplicate by Metabolic Solutions (Nashua, NH). Total body water (TBW) at time of initial capture was estimated from the  $^{18}\text{O}$  dilution space by using equation (17.11) of Speakman (1997). The percentage of body water was assumed to remain constant during the measurement period. Water influx rate (WIR) was calculated using the equation of Nagy and Costa (1980), and rates of  $\text{CO}_2$  production were calculated using equation (7.17) of Speakman (1997). Background levels of isotopes from each animal were subtracted from values before calculations. To calculate energy expenditure, we used an energy equivalence of  $21.7 \text{ J mL}^{-1} \text{ CO}_2$  (Nagy 1983). Body composition of mountain beavers was assessed using TBW measurements and assuming that fat-free mass had a hydration state of 72.4% (Pace and Rathburn 1945). A water economy index (WEI;  $\text{mL H}_2\text{O influx kJ}^{-1}$  metabolized) was calculated as the ratio of WIR ( $\text{mL H}_2\text{O d}^{-1}$ ) to FMR ( $\text{kJ d}^{-1}$ ; Nagy and Peterson 1988).

For animals that live in burrows, rebreathing of labeled expired  $\text{CO}_2$  is a potential source of error in estimates of FMR (Nagy 1983). However, because mountain beaver burrows are shallow, extending  $<0.8$  m belowground (Fellers et al. 2004), sufficient exchange of burrow air with the atmosphere to prevent significant rebreathing of labeled air was assumed (Williams et al. 1997, 2002). Mountain beavers exhibit coprophagia, which may lead to the reingestion of labeled water to some unknown extent. This would lead to an underestimation of isotopic turnover and, thus, an underestimation of water flux.

### *Hormone Analysis*

All hormone assays were run using the initial-capture blood samples. Blood samples for aldosterone analysis were taken into chilled serum vacutainers, placed on ice in the field, returned to the laboratory within 2 h of sampling, centrifuged, and then stored at  $-80^\circ\text{C}$  until analyzed. Blood samples for arginine-vasopressin (AVP) analysis were collected similarly, using vacutainers containing EDTA and with the addition of 100  $\mu\text{L}$  of protease inhibitor cocktail (Sigma) per 1 mL blood. Hormone concentrations were measured by radioimmunoassay using commercially available kits: AVP (Phoenix Pharmaceuticals, Belmont, CA) and aldosterone (DPC, Los Angeles, CA). For measurements of AVP, 1.0-mL aliquots were extracted using C-

18 columns (Prep-Sep, Fisher Scientific, Fair Lawn, NJ), as previously described (Ortiz et al. 2006). Serially diluted samples displayed significant parallelism with the standard curve for AVP and aldosterone. All samples were analyzed in duplicate and run in a single assay with intra-assay percent coefficient of variability of less than 8% for all assays.

## Results

The initial sample consisted of five adult postreproductive females and two adult males. Five animals, four females and one male, were recaptured. Mass, TBW, body composition, and interval between captures for each animal are shown in Table 1. The mean change in mass over the measurement interval was a decrease of  $1.2\% \pm 1.5\%$ . The ratio of  $^3\text{H}$  water dilution space to  $^{18}\text{O}$  water dilution space was  $1.01 \pm 0.01$ .

WIR, FMR, and WEI are shown in Table 1. Mean water flux was  $210 \pm 32 \text{ mL H}_2\text{O d}^{-1}$ . This represented  $293 \pm 46 \text{ mL H}_2\text{O d}^{-1} \text{ kg}^{-1}$ , or  $45.0\% \pm 6.3\% \text{ TBW d}^{-1}$ . WIR did not vary significantly with body mass ( $r^2 = 0.46$ ,  $P = 0.21$ ). FMR of subjects averaged  $1,488 \pm 486 \text{ kJ d}^{-1}$ . FMR was significantly related to body mass (Fig. 1). The intraspecific scaling exponent for FMR was 1.54. WIR did not vary significantly with FMR ( $r^2 = 0.59$ ,  $P = 0.13$ ). WEI decreased significantly with body mass (Fig. 2). Plasma hormone, metabolite, and electrolyte concentrations for all subjects are shown in Table 2. Plasma blood urea nitrogen to creatinine ratios (U : C), an index of glomerular filtration rate (GFR), were high, averaging  $64 \pm 34$ . AVP concentrations were significantly negatively related to WIR (Fig. 3). U : C increased significantly with AVP concentrations (Fig. 4). AVP concentrations were not related to WEI or serum electrolytes ( $P > 0.05$ ). Aldosterone concentrations were not related to WIR, WEI, or serum electrolyte concentrations ( $P > 0.05$ ).

## Discussion

### *Water Flux Compared with That of Other Mammals*

Measured water flux rates of mountain beavers can be compared with predictions from several allometric equations. Nagy and Peterson (1988) derived an equation that predicts water flux rate for eutherian mammals in the field and is based on 115 data points from 42 species (water flux =  $0.326[\text{body mass}]^{0.818}$ , where water flux is in  $\text{mL H}_2\text{O d}^{-1}$  and body mass is in g). This equation predicts a mean water flux rate of  $71.3 \text{ mL d}^{-1}$  for the subjects in our study. Measured water flux rates averaged 297% of predicted values. Nagy and Peterson (1988) also derived an equation for herbivorous eutherian mammals based on 28 data points from eight species (water flux =  $0.708[\text{body mass}]^{0.795}$ ). This equation predicts a mean water flux rate of  $140.2 \text{ mL d}^{-1}$  for the mountain beavers. Measured water flux rates averaged 159% of values predicted for similar-sized eutherian herbivores. This predictive equation did contain data from two species found in hygric habitats but did not allow a separate equation based on habitat to be derived. Studies on animals living in wet habitats (Williams and Ridpath 1982; Grenot et al. 1984; Berteaux and Thomas 1999) have revealed water flux rates that are 210%–289% of those predicted for similar-sized herbivorous eutherian mammals. In comparison, the mass-controlled water flux values from this study are somewhat smaller. However, studies on rodents have, in general, suggested lower rates of water flux in that group. Morris and Bradshaw (1981) derived an allometric equation predicting water flux specifically for rodents (water flux =  $0.21[\text{body mass}]^{0.9}$ ). This equation predicts a water flux rate of  $79 \text{ mL d}^{-1}$  for the mountain beavers. Measured water flux rates averaged 269% of values predicted for similar-sized rodents. Our findings support the indirect conclusions on field water economy in *Aplodontia* inferred from anatomical and laboratory studies.

Table 1: Total body water (TBW), body composition, water influx rate (WIR), field metabolic rate (FMR), and water economy index (WEI) in mountain beavers

Animal	Mass (g)	TBW (mL)	Body Fat (%)	Interval (d)	WIR ( $\text{mL d}^{-1}$ )	FMR ( $\text{kJ d}^{-1}$ )	WEI ( $\text{mL kJ}^{-1}$ )
Mb1	787	497	13.5	6.91	200	1,502	.13
Mb2	910	589	11.3	5.79	248	2,246	.11
Mb3	770	489	12.9	4.94	207	1,530	.14
Mb4	622	412	9.3	6.95	230	1,213	.19
Mb5	549	369	7.9	6.94	164	948	.17
Mb6	494	326	9.7				
Mb7	812	529	10.7				
Mean	690	459	10.8	6.41	210	1,488	.15
SD	146	93	2.0	.85	32	486	.03

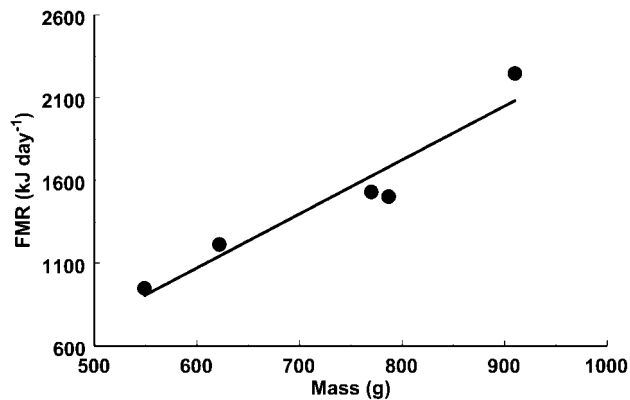


Figure 1. Relationship of body mass to field metabolic rate (FMR) in five adult Point Reyes mountain beavers. FMR was measured using the doubly labeled water method.  $FMR = -886 + 3.26(\text{mass})$  (where FMR is in  $\text{kJ d}^{-1}$  and mass is in g);  $r^2 = 0.92$ ,  $F_{1,3} = 34.9$ ,  $P < 0.001$ .

#### FMR Compared with That of Other Mammals

Mountain beavers in this study exhibited extremely high FMR. Nagy et al. (1999) derived an allometric equation for predicting the FMR of herbivores based on 26 species of herbivores ( $FMR = 7.94[\text{mass}]^{0.646}$ , where FMR is in  $\text{kJ d}^{-1}$  and mass is in g) and for eutherian mammals based on 58 species ( $FMR = 4.21[\text{mass}]^{0.772}$ ). These equations predict values for FMR of 561 and 682  $\text{kJ d}^{-1}$ , respectively. Values in our study were 265% and 218% of these predicted values, respectively. In contrast, McNab (1979) concluded that, in general, fossorial mammals with body weight  $>100$  g have low basal rates of metabolism. However, McNab's data on *Aplodontia* thermal energetics give some insight into the high FMR measured in this study. McNab (1979) measured a standard thermal conductance and a lower critical temperature of  $26.5^\circ\text{C}$  for mountain beavers. Although we lack burrow temperature data for the study period, April and May burrow temperatures at the study site in 1996 and 1997 averaged  $12.0^\circ\text{C}$  (Fellers et al. 2004). This environmental temperature resulted in metabolic rates that were  $\sim 2.4$  times the basal metabolic rate in the laboratory (McNab 1979). Our average FMR was 2.4 times the basal metabolic rate estimated by McNab (1979). The high intraspecific mass-scaling exponent for FMR in our sample (1.54) is likely influenced by our small sample size and the fact that the largest animal was the only male in the sample.

The high FMR in our sample resulted in low WEI, despite high rates of water flux. Herbivores that consume green forage without drinking are expected to exhibit WEI ranging from 0.15 to 0.26  $\text{mL H}_2\text{O kJ}^{-1}$  (Nagy and Peterson 1988). Values measured in this study (0.15  $\text{mL H}_2\text{O kJ}^{-1}$ ) are at the lower end of that range, suggesting that although mountain beavers had high water flux rates, they ingested relatively low rates of water for their metabolic rates. A DLW study on voles living in hygric habitats reported summer metabolic rates that were

123% of those predicted by the above herbivore equation and revealed consistent and high WEIs across seasons, despite seasonal variability in energy expenditure and water flux (Berteaux and Thomas 1999). The vole study suggested that energy expenditure for thermoregulation influences rates of water flux by increasing water intake from food and from metabolic water production. Mountain beavers exhibited significantly lower ratios to predicted values of water flux than the voles (159% vs. 297%), despite significantly higher ratios of FMR to predicted values (218% vs. 123%). Our measurements were made in late spring, when significant time had passed since the last rainfall, and all free environmental water came from early morning fog drip. Seasonal effects on water flux, metabolism, and water economy await further investigation.

The high FMR and low WEI in mountain beavers suggest a potential for increased importance of metabolic water production to overall water economy. The catabolism of nutrients containing hydrogen produces oxidative water (Schmidt-Nielsen 1964). Whether net water gain can be derived from this process depends on humidity and diet composition. In humid environments, net water gains are usually associated with metabolism of carbohydrates and fats, while protein metabolism results in a large loss of water (Frank 1988). The high C:N ratios associated with ferns and forbs (Robbins 1983), the preferred mountain beaver diet, suggest net gain of water through metabolism. If we assume 100% metabolism from starch and 0.0316  $\text{g H}_2\text{O kJ}^{-1}$  (Costa 1987), metabolic water production could account for approximately 22% of daily water flux for an average mountain beaver. The low WEI in our sample suggests that despite potential deficits in urinary concentrating ability, mountain beavers minimize alternate routes of water loss to the environment. The moist habitat and burrow environment used by this species may help to minimize total evaporatory water loss (TEWL) compared with that experienced

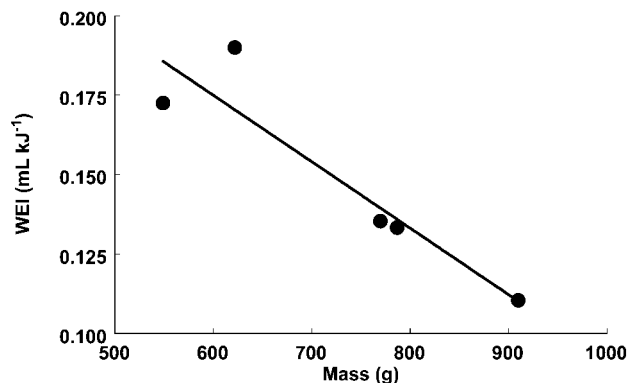


Figure 2. Relationship of water economy index (WEI) to body mass in mountain beavers. WEI was calculated as water interflux rate ( $\text{mL d}^{-1}$ ) divided by field metabolic rate ( $\text{kJ d}^{-1}$ ).  $WEI = -0.0002(\text{mass}) + 0.301$  (where mass is in g);  $r^2 = 0.86$ ,  $F_{1,3} = 18.5$ ,  $P = 0.02$ .

Table 2: Osmoregulatory hormone electrolyte, blood urea nitrogen (BUN), and creatinine (Cr) concentrations in mountain beaver plasma

Animal	AVP (pg mL <sup>-1</sup> )	Ald (pg mL <sup>-1</sup> )	Na <sup>+</sup> (mEq L <sup>-1</sup> )	K <sup>+</sup> (mEq L <sup>-1</sup> )	Cl <sup>-</sup> (mEq L <sup>-1</sup> )	BUN (mg dL <sup>-1</sup> )	Cr (mg dL <sup>-1</sup> )	U : C
Mb1	43	487	145	2.7	103	43	.6	72
Mb2	23	513	146	3.7	107	16	.4	40
Mb3	25	357	146	3.3	107	20	.4	60
Mb4	16	140	133	4.3	93	5	.3	17
Mb5	55	995	137	4.1	102	34	.4	85
Mb6	54	249	136	3.4	90	37	.3	123
Mb7	35	556	136	2.4	102	39	.9	54
Mean	36	471	140	3.4	101	29	.5	64
SD	15	276	6	.7	7	16	.2	34

Note. U : C is the ratio of BUN to creatinine. Aldosterone (Ald) and arginine-vasopressin (AVP) were measured using commercial kits containing antibodies to mouse hormones raised in rabbits.

by other herbivores. This idea is supported by the strong inverse relationship between body mass and WEI in our sample. The three largest animals had WEI well below the lower limit of expected values.

#### Free Water Requirements

Although mountain beavers were observed to drink copious water in captivity (Schmidt-Nielsen and Pfeiffer 1970), they also varied this behavior with food water content (Nungesser and Pfeiffer 1965). It is not known whether they usually drink in the field. Lean body compositions measured in this study suggest a reduced ability to use stored body reserves for metabolic water production. While the varied diet of the mountain beaver precludes exact calculations, we can estimate the extent of nonfood water intake based on average values for similar plants. If we assume an energy density of 17.6 kJ g<sup>-1</sup> dry weight (Golley 1961) and an assimilation efficiency of 80% (McNab 2002), an average mountain beaver in our sample would need to ingest 106 g of dry food per day. In order to meet the remaining 78% of WIR not provided by metabolic water production, an average mountain beaver would need to ingest an additional 163 mL of water, requiring a minimal plant water content of 61%. While water and energy contents of plants may covary, this level of water content is reasonable, given the mountain beaver's preference for succulent plants. Our measurements suggest that high rates of metabolism, and the high rates of food intake and metabolic water production they necessitate, allow mountain beavers to meet their high rates of water flux through ingestion of preformed water in food. This is consistent with the findings of Fisler (1965), who was able to maintain captive individuals that did not drink water for months.

#### Hormonal Regulation

Previous studies have suggested that mountain beaver kidneys have a reduced or absent response to vasopressin (Greenbaum and Dicker 1963). However, administered vasopressin weakly reduced urine flow and plasma osmolality and increased urine : plasma osmolality ratios to levels similar to those seen in response to short-term fasting (Dicker and Eggleton 1964). Very large doses of vasopressin produced only slightly stronger effects (Dolph et al. 1962). These responses to vasopressin were small when compared with those of other rodents. AVP values in mountain beavers ( $36.6 \pm 15.4$  pg mL<sup>-1</sup>) were significantly higher than those reported in captive rats (10.4 pg mL<sup>-1</sup>; Yamaguchi et al. 1982) but significantly lower than those reported in some desert rodents (e.g., 162 pg mL<sup>-1</sup>; Baddouri and Quayou 1991). Vasopressin's primary role is stimulating the formation of aquaporin-2 water channels in the apical membrane of the collecting duct, thus increasing its permeability (Nielsen et al.

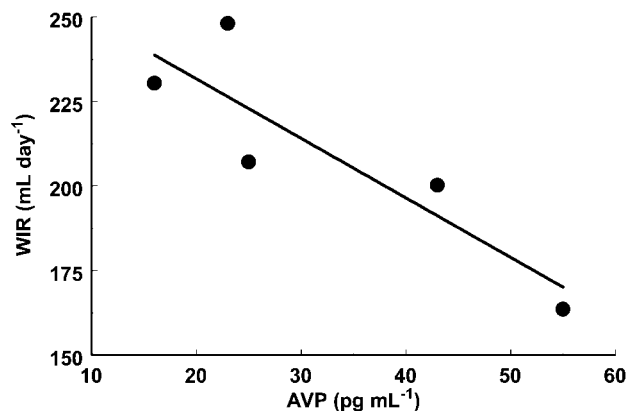


Figure 3. Changes in water influx rate (WIR) with plasma arginine-vasopressin (AVP) concentrations.  $WIR = 267 - 1.76(AVP)$ ;  $r^2 = 0.78$ ,  $F_{1,3} = 10.7$ ,  $P = 0.04$ .

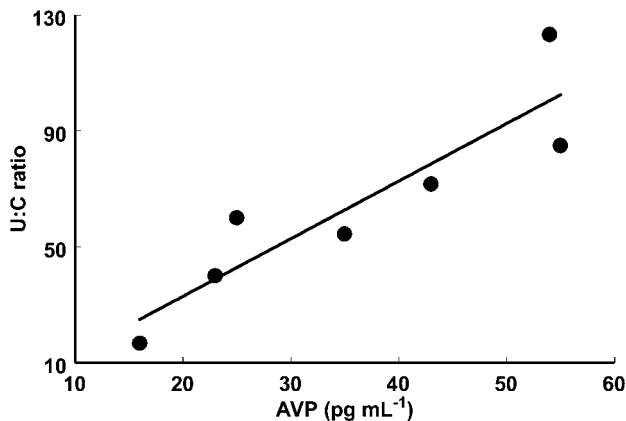


Figure 4. Changes in plasma blood urea nitrogen ( $\text{mg dL}^{-1}$ ) to creatine ( $\text{mg dL}^{-1}$ ) ratio (U : C) with plasma arginine-vasopressin (AVP) concentrations.  $\text{U : C} = -6.8 + 2.0(\text{AVP})$ ;  $r^2 = 0.81$ ,  $F_{1,3} = 21.5$ ,  $P = 0.006$ .

2002). The effectiveness of this increased permeability in concentrating urine would be limited by lack of a significantly hypertonic medulla, as suggested by the anatomy of the mountain beaver kidney. However, despite the moderate response to injected vasopressin relative to other rodents and low resultant urine osmolality, the threefold increase in urine osmolality reported by Dicker and Eggleton (1964) may significantly affect urinary water loss in mountain beavers. Studies have also suggested important roles for vasopressin in social behavior in rodents (Wang et al. 2000) and that vasopressin may serve to link environmental water stress to reproductive cycles (Shanas and Haim 2004). The strong negative relationship between AVP concentrations and WIR suggests an important role for AVP in reducing urinary water loss in mountain beavers. AVP has been implicated in the elevation of GFR in rats (Roald et al. 2000). Plasma U : C ratio, which can be an indicator of altered GFR (Duarte and Preuss 1993), showed a strong positive relationship with AVP, suggesting that a similar relationship is possible in mountain beavers.

Captive mountain beavers placed on low-protein diets exhibited urine urea levels similar to those of animals on high-protein diets but exhibited significantly lower levels of urine sodium (Schmidt-Nielsen and Pfeiffer 1970). Saline infusion experiments suggested that this occurred from reabsorption of sodium, with resulting water reabsorption and urea concentration (Schmidt-Nielsen and Pfeiffer 1970). Thus, the ability to increase urinary osmolality in response to dehydration in captive mountain beavers may be due to increased sodium reabsorption induced by aldosterone. Plasma aldosterone levels in our study were high and varied widely from animal to animal. Time in the cage may have also influenced hydration status and, thus, levels of osmoregulatory hormones.

In conclusion, we measured high rates of water flux and

energy expenditure in mountain beavers when compared with other herbivorous mammals. These findings are consistent with anatomical findings and distributional limits related to moisture. Low WEI despite high rates of water flux suggests that urinary concentrating ability may not be the primary factor in determining water flux in this species and that TEWL is minimized. Our data suggest an important role of AVP in regulating water flux in mountain beavers, despite their renal anatomy. Because of high rates of energy expenditure and low WEI, mountain beavers can likely meet their water needs through metabolic water production and preformed water in food and can thus remain in water balance without access to free water. Future comparative studies on the seven subspecies of *Aplodontia rufa* relative to habitat moisture, thermal regimes, and seasonal effects will give increased insight into the factors driving rates of water flux and energy expenditure.

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